



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 2380

Drugs of Abuse in Human Hair II

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining morphine (MOR), codeine (COD), 6-monoacetylmorphine (MAM), and tetrahydrocannabinol (THC) in human hair and similar materials. This SRM can also be used for quality assurance when assigning values to in-house control materials. The SRM is a drug-free human hair material, to which drugs of abuse have been incorporated. A unit of SRM 2380 consists of one bottle containing approximately 100 mg of hair segments.

Certified Concentration Values: Analyses for value assignment were performed at NIST. All certified values are the equally weighted means of the measurements made by two independent methods at NIST for a given analyte; the associated uncertainties are expressed at the approximate 95 % level of confidence [1,2,3]. Values are reported on an as-received basis in mass fraction units. The certified concentration values of the target compounds in SRM 2380 are provided in Table 1.

Expiration of Certification: The certification of this SRM lot is valid until **01 January 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by M.J. Welch of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by L.T. Sniegowski and S. S-C. Tai of the NIST Analytical Chemistry Division.

Statistical analysis was provided by N.-f. Zhang of the NIST Statistical Engineering Division.

The support aspects involved with the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Group by J.C. Colbert.

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NOTICE AND WARNING TO USERS

Storage: The SRM should be stored under refrigeration at temperatures between 4 °C to 8 °C.

Warning: For laboratory use only.

INSTRUCTIONS FOR USE

A quantity of hair appropriate for a particular analysis should be withdrawn and weighed on an analytical balance prior to extraction and analysis. Particular care must be exercised for determinations of morphine and monoacetylmorphine (MAM), because of the possible conversion of significant quantities of MAM to morphine during the sample preparation. Effects can be minimized by spiking the sample with isotope-labeled internal standards at levels near to those of the unlabeled analytes prior to extraction.

PREPARATION AND ANALYSIS¹

Preparation: SRM 2380 is human hair from a single individual. The hair was manually cut into short segments and then soaked for 17 days in water:dimethylsulfoxide (1:1) containing the target analytes. The hair was removed from the mother liquor, rinsed several times with methanol and allowed to air dry.

NIST Analyses For the Target Drugs of Abuse: Two independent methods were used at NIST for each of the analytes. One method involved measurements by gas chromatography/mass spectrometry (GC/MS); the other method involved measurements by liquid chromatography/mass spectrometry (LC/MS). For the GC/MS method for the opiates [4], hair samples were spiked with deuterated analogs of each of the analytes and then extracted with 0.1 M HCl at 45 °C for 24 h. The extracts were neutralized and put through commercial solid-phase extraction cartridges designed for urine drug testing following the manufacturer's directions. After the eluants were collected and dried, codeine, morphine, and monoacetylmorphine were dissolved in N,O-bis(trimethylsilyl)-acetamide (BSA) and analyzed together. For the GC/MS measurements of tetrahydrocannabinol (THC), the hair samples were treated with 1 M NaOH and heated at 95 °C for 30 min. The mixture was then acidified to pH 4-5 and put through a solid-phase extraction cartridge. The eluant was treated with BSA to derivatize the THC for GC/MS analysis. For both methods, the GC/MS was equipped with a 30 m low polarity GC column and measurements were performed using electron ionization and selected ion monitoring. For the LC/MS method for the opiates, hair samples were spiked with deuterated analogs of each of the analytes and extracted with 0.1 M HCl at 45 °C for 24 h. The extracts were neutralized to pH 6.0 and put through solid-phase extraction cartridges that were different from those used for GC/MS. No derivatization was necessary and the three opiate compounds were measured together in a single run. The LC was equipped with a deactivated C18 column and compounds were eluted using a gradient mobile phase of 0.1% acetic acid in water and methanol. For the LC/MS measurement of THC, the hair samples were treated with 1 M NaOH and heated at 95 °C for 30 min. After the pH of the samples was adjusted to pH 3-4, THC was extracted with hexane:ethyl acetate (9:1 v:v). This extract was then dried and reconstituted in methanol for analysis by LC/MS. This method used a different deactivated C18 column, but the mobile phase was the same as used for the opiates. The electrospray ionization conditions were optimized for each analyte. For both GC/MS and LC/MS methods, three sets of samples were measured for all of the analytes. Each set consisted of two samples each from three bottles of hair. Calibration standards were run before and after the samples, and a standard curve was generated by a least squares fit of all of the standards measurements for a particular set.

Homogeneity Assessment: For the sample sizes used for the certification measurements (20 mg to 25 mg), there was no evidence of within-bottle inhomogeneity. There also was no correlation between fill order and concentrations measured for any of the analytes.

¹Certain commercial materials and equipment are identified to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

Value Assignment: For each analyte, the equally weighted means from the two measurement methods were combined using the “Bayesian BOB” statistical approach [3]. The standard uncertainty was calculated by taking the square root of the sum of the squares of the uncertainty from the Bayesian BOB calculation, an uncertainty component for the purity of the reference compound used, and an uncertainty component for the possibility of incomplete extraction. The standard uncertainty was multiplied by a coverage factor of two to get the expanded uncertainty.

Table 1. Certified Concentrations^a (Mass Fractions) for Analytes in SRM 2380

Analyte	Mass Fraction (ng/mg)
Codeine	9.82 ± 0.70
Morphine	10.54 ± 0.68
6-Monoacetylmorphine	2.71 ± 0.30
Tetrahydrocannabinol	0.99 ± 0.10

^a Each certified concentration value, expressed as a mass fraction for the material (as received), is an equally weighted mean from the combination of results from two analyses by NIST. The uncertainty in the certified values, calculated according to the method described in the ISO Guide [1,2], is expressed as an expanded uncertainty, U . The expanded uncertainty is calculated as $U = k u_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-method and within-method components of uncertainty. The coverage factor, $k = 2$, corresponds to an approximate 95 % confidence interval for each analyte.

REFERENCES

- [1] *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st Ed., ISO, Geneva, Switzerland, (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC (1994); <http://physics.nist.gov/Pubs/>.
- [2] Schiller, S.B.; Eberhardt, K.E.; *Combining Data from Independent Chemical Analysis Methods*; Spectrochim. Acta; 46B, No. 12, pp. 1607-1613 (1991).
- [3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H-k.; Vangel, M.G.; Yen, J.H.; Ahang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Ntl. Inst. Std. Techno.; Vol. 105, pp. 571-579 (2000).
- [4] Welch, M.J.; Sniegowski, L.T.; Allgood, C.C.; Habram, M.; *Hair Analysis for Drugs of Abuse: Evaluation of Analytical Methods, Environmental Issues, and Development of Reference Materials*; J. Anal. Toxicol; Vol. 17, pp. 389-398 (1993).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Group at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet <http://www.nist.gov/srm>.